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Identification of JC Virus Variants in Multiple Tissues of Pediatric and Adult PML Patients

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The transcriptional control region (TCR) of JC virus (JCV), the causative agent of progressive multifocal leukoencephalopathy (PML), undergoes rearrangement during replication of the virus in its human host. The mechanism by which viral promoter/enhancer sequences are deleted and duplicated within the TCR of the archetype form of JCV is not understood, but it is hypothesized that the generation of JCV variants with rearranged TCRs contributes to the virus's pathogenic potential. In a recent study of a pediatric PML patient, we detected extensive rearrangement of the JCV TCR in multiple tissues, and the archetype TCR was amplified from sites other than the kidney. These findings differed from those of previous studies that had examined tissues from adult PML patients. Since exposure to JCV usually occurs early in life, it is likely that some pediatric cases of PML arise as the result of a primary infection, whereas adult cases of PML are thought to result from the reactivation of an infection suffered as an immunocompetent child. To investigate further whether rearrangement of the JCV TCR is affected by the host's age and immune status at the time of exposure, a second pediatric patient and two adult PML patients were examined. As in our first study, multiple tissues were found to contain JCV DNA; however, fewer rearranged variants were detected. In one adult patient, related rearranged variants were detected in the brain, while archetype JCV was found in the other tissues. Based on differences in their VP1 sequences, these two forms represented different JCV genotypes, indicating that this patient had suffered a dual infection. The relevance of these findings to the rearrangement process that alters the JCV TCR is discussed. ***J. Med. Virol. 58:79–86, 1999.***

KEY WORDS: polyomavirus; JCV genotype; PCR; immunocompromised host

INTRODUCTION

JC virus (JCV) causes an asymptomatic infection in more than 80% of the human population [Padgett and Walker, 1973; Taguchi et al., 1982]. In severely immunocompromised individuals, a primary or persistent infection may lead to the fatal demyelinating brain disease progressive multifocal leukoencephalopathy (PML). JCV variants have been isolated from both immunocompetent and immunocompromised individuals [Martin et al., 1985; Loeber and Dörries, 1988; Markowitz et al., 1991; Yogo et al., 1991b; Tominaga et al., 1992; White et al., 1992; Ault and Stone, 1993; Dörries et al., 1994; Kitamura et al., 1994b] and classification schemes have been developed to assign viral isolates to different groups.

One such scheme, based on sequence variations within the JCV transcriptional control region (TCR), has led to the recognition of two forms of virus, called archetype and rearranged type (or PML type) JCV. The archetype TCR contains one copy of the promoter/enhancer, while the rearranged TCRs exhibit duplications and deletions within the promoter/enhancer [Yogo et al., 1990, 1991a; White et al., 1992; Ault and Stoner, 1993]. Archetype JCV, which is suspected of being the form that circulates in the population [Yogo et al., 1990; Flægstad et al., 1991; Ault and Stoner, 1993], appears to establish a persistent infection in the kidney; it has been isolated from the kidney and urine of both immunocompetent and immunodeficient individuals [Loeber and Dörries, 1988; Yogo et al., 1990; Flægstad et al., 1991; Markowitz et al., 1991; Tominaga et al., 1992; White et al., 1992; Kitamura et al., 1994a; Kunitake et al., 1995; Agostini et al., 1996b; Guo et al., 1996; Sugimoto et al., 1997]. On the other

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TABLE I. Patient Information

Patient	Sex	Resident of	Disease duration[a]	Age at time of death, years	Underlying illness[b]	Reference
AH	M	Sweden	3 mo	11	SCID	Zu Rhein et al. [1978]
AT	F	United States	2 mo	56	Lymphocytic interstitial pneumonitis	Scully et al. [1980]
AC	F	United States	3.5 mo	55	SLE	Jones et al. [1982]

[a]Duration of disease represents the interval of time between diagnosis of PML and death.
[b]SCID, severe combined immunodeficiency syndrome; SLE, systemic lupus erythematosus (in remission at time of PML diagnosis).

hand, regulatory region variants have been isolated from the brain and lymphocytes of individuals with and without PML and from the kidney of PML patients [Martin et al., 1985; White et al., 1992; Ault and Stoner, 1993; Dörries et al., 1994; Kato et al., 1994; Yogo et al., 1994; Elsner and Dörries, 1998]. In most individuals, one predominant rearranged form is identified [White et al., 1992; Ault and Stoner, 1993; Kato et al., 1994; Agostini et al., 1997b; Elsner and Dörries, 1998].

A second classification scheme, involving nucleotide polymorphisms found within the coding region for the JCV capsid protein, VP1, has been used to group JCV isolates into three genotypes [Agostini et al., 1997c, 1998]. These genotypes are associated with distinct human populations; type 1, 2, and 3 viruses circulate in peoples of European, Asian, and African descent, respectively [Agostini et al., 1995, 1996a, 1996b, 1997c, 1998]. Other investigators have proposed similar genotyping schemes based on these and other nucleotide polymorphisms in variant JCV genomes [Iida et al., 1993; Guo et al., 1996; Sugimoto et al., 1997]. Interestingly, type 2 genomes have been reported to be disproportionally represented in PML brain tissue relative to their overall incidence in the human population [Agostini et al., 1997a].

Recently we characterized JCV TCR variants present in the tissues of a pediatric PML patient [Newman and Frisque, 1997]. PML rarely occurs in children, and in the few cases that have been reported, the victim had a severe, underlying immunocompromising condition [Zu Rhein et al., 1978; Redfearn et al., 1993; Katz et al., 1994]. In our PCR study of a 5-year-old PML patient with severe combined immunodeficiency disease (SCID), multiple rearranged DNAs were amplified from brain, liver, lung, spleen, lymph node, and coeliac plexus tissue. The degree of genomic rearrangement was much more extensive than described in earlier reports [Martin et al., 1985; White et al., 1992; Ault and Stoner, 1993; Kato et al., 1994]. Furthermore, the archetype form was not confined to the kidney and urine but was isolated from brain and lymph node tissue as well.

It has been suggested that this pediatric case of PML arose as a consequence of the primary infection, rather than as a reactivation of a persistent infection [Grinnell et al., 1983b]. We speculated that unchecked replication of JCV in this SCID patient at the primary site of infection resulted in the generation of multiple variants which were then transported to numerous secondary sites via infected lymphocytes. The present study describes our efforts to determine whether the broad distribution of archetype and rearranged JCV variants within the body is unique to pediatric PML patients or whether it also occurs in the more general case of adult PML. Finally, we report the genotypes of the JCV genomes detected in these individuals and demonstrate that in one case a dual infection with type 1 and type 2 JCV had occurred.

MATERIALS AND METHODS

Human Tissue Samples

The PML tissues analyzed in this study were provided by Dr. Duard Walker (University of Wisconsin). Specimens were received either as frozen blocks of tissue or as homogenates. Patient data is shown in Table I, and each patient is identified by their initials (AH, AT, and AC). JCV DNA sequences amplified and cloned from these patients' tissues were named JCV(AH), JCV(AT), and JCV(AC). In earlier studies, viruses had been isolated from the diseased brains of patients AH and AT and were called JCV(Mad 10) and JCV(Mad 11), respectively [Grinnell et al., 1983a; Martin et al., 1985].

Prior to conducting PCR analysis, individual fragments (approximately 8 mm^3) of frozen tissue were placed into 600-μl double-locking microfuge tubes (Robbins Scientific, Sunnyvale, CA). These samples were then digested with 105 μl of tissue digestion buffer and processed as described previously [Newman and Frisque, 1997]. The brain homogenates from patients AH and AT were digested by combining 50 μl of the homogenate with 55 μl of tissue digestion buffer.

PCR Precautions and Controls

A number of procedures were adopted as described previously [Newman and Frisque, 1997] to minimize the risk of contamination. Several negative controls were included to monitor this potential problem. The sensitivity of each reaction was also examined with positive controls as reported elsewhere [Newman and Frisque, 1997]. Positive signals were detected routinely with $<10^2$ input molecules of DNA; some variation in sensitivity was observed depending on the primer pair utilized in the reaction. In addition to these controls, 10^6 molecules of cloned viral DNA were added to an aliquot of digested tissue to determine whether inhibitors of the PCR assay were present in the processed samples.

TABLE II. Nucleotide Sequence of PCR Primers, Probes, and Sequencing Primers

Primer name	Sequence 5′→3′	Nucleotide positions
JRR1	CCTCCACGCCCTTACTACTTCTGAG	5086–5110[a]
JRR2	GTGACAGCTGGCGAAGAACCATGGC	298–274[a]
JRR27	CTCCCTATTCAGCACTTTGTCC	4990–5011[a]
JRR28	TCCAGGTTTTACTAACTTTCACAGA	331–307[a]
JLP15	ACAGTGTGGCCAGAATTCCACTACC	1710–1734[a]
JLP16	TAAAGCCTCCCCCCAACAGAAA	1924–1902[a]
M13 rev	CAGGAAACAGCTATGAC	205–221[b]
T7 promoter	TAATACGACTCACTATAGGG	381–362[b]

[a]Nucleotide numbering for JCV(Mad1) is from Frisque et al. [1984].
[b]Nucleotide numbering for vector pCR 2.1 is from the Original TA Cloning Kit Manual (Invitrogen).

PCR Conditions

The sequences of the PCR primers utilized in this study are listed in Table II. Each PCR assay was composed of the following: 1 μl of digested tissue, Expand HF buffer (Expand High Fidelity PCR System, Boehringer Mannheim, Indianapolis, IN), 3.0 units of a Pwo/Taq DNA polymerase mixture supplied with the Expand High Fidelity kit, 200 μM each of dATP, dGTP, dCTP, and dTTP (Pharmacia Biotech, Uppsala, Sweden), and either 0.5 μM each of primers JRR1 and JRR2 to amplify the regulatory region [White et al., 1992; Newman and Frisque, 1997], or 0.5 μM each of primers JLP15 and JLP16 to amplify a portion of the VP1 coding region [Agostini et al., 1997c]. A HotWax Mg^{++} bead (Invitrogen, Carlsbad, CA) containing $MgCl_2$ (final concentration 1.5 mM) was added to each reaction mixture, and the final volume was brought to 50 μl with double distilled H_2O. Positive controls for reactions involving primers JRR1/JRR2 contained 10^1 to 10^6 input molecules of recombinant JCV (Mad1) DNA (pM1TCR1A; [Frisque et al., 1984]). Amplification was carried out using a MJR PTC-200 DNA Thermocycler (MJ Research, Watertown, MA). Cycling conditions for reactions using primer pair JRR1/JRR2 were described previously [Newman and Frisque, 1997]. For reactions involving primer pair JLP15/16, a total of 45 cycles was used, which included a denaturation step at 94°C for 30 sec followed by a combined annealing-extension step at 63°C for 1 min [Agostini et al., 1997c].

A nested PCR strategy was adopted for reactions involving lung, lymph node, and liver tissue from patient AC and spleen from patient AH to increase product yield and cloning efficiency. JRR27/JRR28 was used as the outer primer pair for the first PCR reaction, and 1 μl of the reaction products was then amplified with JRR1/JRR2 acting as the inner primer pair. A parallel reaction with the inner pair alone was included for all samples. The Expand High Fidelity PCR System and the hotstart technique as described above were used for the nested PCR. Cycling conditions were the same as those used in the unnested reaction with primer pair JRR1/JRR2 [Newman and Frisque, 1997], except that 30 and 25 cycles were used for the first and second reactions, respectively.

Gel Electrophoresis

PCR products were electrophoresed on composite minigels containing 0.7% molecular biology certified agarose (Eastman Kodak, Rochester, NY), 2% NuSieve GTG agarose (FMC BioProducts, Rockland, ME), TBE buffer, and 0.5-μg/μl ethidium bromide. Gels were electrophoresed for 45 to 60 min at 130 volts, and the DNAs were visualized on a short wavelength UV light box and photographed with the EagleEye II System (Strategene, La Jolla, CA).

DNA Cloning, Isolation, and Quantitation

PCR products were cloned into the TA Cloning vector pCR 2.1 (Invitrogen). Ligation and transformation reactions were carried out as described in the Original TA Cloning Kit protocol. Recombinant DNA was isolated from selected ampicillin-resistant bacterial transformants using the Wizard Plus Minipreps Kit (Promega, Madison, WI) and digested with *BstXI* to identify clones containing JCV DNA. The size of the *BstXI*-generated DNA fragment containing the PCR insert was compared to the BioMarker Low molecular weight marker (BioVentures, Murfreesboro, TN) to identify recombinants for sequence analysis. A TKO 100 minifluorometer (Hoefer Scientific Instruments, San Francisco, CA) was used to quantitate the DNA in preparation for sequencing.

DNA Sequencing and Analysis

Selected clones were sequenced by the Nucleic Acid Facility at The Pennsylvania State University Biotechnology Institute using an ABI 377 Prism Sequencer (Perkin-Elmer, Oak Brook, IL). Each sample was analyzed using M13rev and T7 primers (Table II), which permitted sequencing of both strands of a PCR product cloned into pCR 2.1. The resulting trace files for each reaction were analyzed and compiled with the Lasergene SeqManII program (DNAStar, Madison, WI) package for Windows NT 4.0 (Microsoft, Redmond, WA).

Cloned JCV TCR sequences were compared to those of the CY archetype strain of JCV [Yogo et al., 1990]. Nucleotide differences between TCR clones were identified by the MegAlign Lasergene program. Cloned JCV VP1 sequences were compared to those of the type 1A and 2B genotypes [Agostini et al., 1997c, 1998] using

this same software program. Genotype determination was based on the scheme proposed by Stoner et al. [Agostini et al., 1995, 1996a, 1996b, 1997c, 1998].

RESULTS

Amplification, Cloning, and Sequencing of JCV DNA Present in Multiple Tissues of Three PML Patients

Most PML patients are infected by JCV early in life [Padgett and Walker, 1973; Taguchi et al., 1982; Walker and Padgett, 1983]. The onset of their demyelinating disease occurs years later after a reactivation of the persistent infection during a severe immunocompromising event. In contrast, pediatric PML likely arises as a consequence of the primary infection in an immunodeficient child [Grinnell et al., 1983b]. In a recent study, we examined one such pediatric case and identified several rearranged forms of JCV distributed in a number of different tissues [Newman and Frisque, 1997]. These findings lead us to suggest that the preexisting impairment of the immune system at the time of the initial infection (the child had SCID) may have permitted unabated replication of the virus and extensive rearrangement of the viral TCR to occur at the primary site of multiplication. To obtain support for this suggestion, we have now amplified JCV regulatory region sequences from the tissues of a second pediatric patient. For comparison, JCV DNA was isolated from two adult PML patients in whom the onset of disease was likely the result of reactivation of a persistent infection. Multiple tissues from the three different patients were examined by standard and nested PCR, and JCV DNA was found in all the specimens. To identify the JCV variants detected in these patients, amplified viral TCR and VP1 sequences were cloned and subjected to sequence analysis.

Identification of Archetype and Rearranged Forms of JCV in Multiple Tissues of Pediatric PML Patient AH

Two identical clones representing the archetype TCR were isolated from the kidney of the 11-year-old PML patient AH (Fig. 1). Both clones have a single copy of the promoter/enhancer and retain the 66-bp region often deleted from rearranged forms of JCV [Yogo et al., 1990; Ault and Stoner, 1993]. A G→A transition is present at nucleotide position 217 relative to the CY archetype reference strain. Nucleotide polymorphisms are known to occur at this site [Yogo et al., 1990], and this particular transition is present in all the TCR clones sequenced in this study.

A single rearranged TCR sequence was amplified from the other tissues available for this patient (Fig. 1). This TCR contains a duplicated promoter/enhancer, and like most rearranged forms already isolated [Martin et al., 1985; Loeber and Dörries, 1988; White et al., 1992; Ault and Stoner, 1993; Kato et al., 1994; Newman and Frisque, 1997], it lacks the 66-bp region. There is some uncertainty as to the position of the deletion boundaries; the endpoints are shown at nucleotide positions 112 and 183 in Figure 1, but assignment of positions 110 and 181 or 111 and 182 as the boundaries would also be consistent with the data. There is some ambiguity in denoting the junction between the first and second copies of the promoter/enhancer as well; alternate positions to those shown in Figure 1 include 204 and 59, 205 and 60, and 206 and 61. In contrast to results obtained for the one other pediatric PML patient examined to date [Newman and Frisque, 1997], only a single rearranged clone was identified in pediatric patient AH.

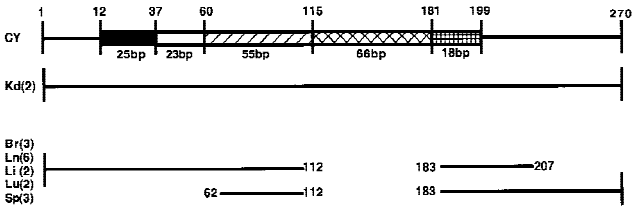

Fig. 1. Structures of the JCV(AH) TCRs derived from multiple tissues of pediatric PML patient AH. Nucleotide numbers represent the positions within the archetype CY sequence [Yogo et al., 1990]. Clones are identified at the left and are followed by a line that is read completely from left to right. A gap within a line represents a deletion of archetype sequence, and parallel lines are multiple copies of that particular sequence. Tissue abbreviations are as follows: kidney (Kd), brain (Br), lymph node (Ln), liver (Li), lung (Lu), and spleen (Sp). The number in parentheses indicate the number of individual clones isolated from that tissue. All clones have a G→A transition at position 217 relative to the CY archetype strain [Yogo et al., 1990].

Identification of a Rearranged Form of JCV in Multiple Tissues of Adult PML Patient AT

Previous studies have generally detected only one or two rearranged forms of JCV in adult PML patients [White et al., 1992; Ault and Stoner, 1993; Kato et al., 1994; Agostini et al., 1997b; Elsner and Dörries, 1998]. In each of these cases, PML was probably the result of the reactivation of an infection that had been established early in life. Based on these results, and in contrast to our earlier finding of multiple rearranged forms of JCV in a 5-year-old PML patient [Newman and Frisque, 1997], we expected to find a unique predominant rearranged form in each of the two adult cases examined in the present study.

TCR sequences obtained from adult patient AT are shown in Figure 2. The same rearranged TCR was found in all the tissues examined, including kidney, and its sequence was identical to that determined for the JCV(Mad11Br) genome cloned directly from the brain of this patient in an earlier study [Grinnell et al., 1983a]. Two additional TCR clones, one from the lung and one from the liver, were obtained from patient AT. These were clearly related to the predominant clone. All three TCR clones contain a deletion in the late leader sequences (between nucleotides 236 and 257; Fig. 2). Minor changes involving the junction between the first and second copies of the promoter/enhancer were noted. Unlike the typical rearranged JCV regulatory region, the TCR sequences amplified from patient AT retain most of the 66-bp region and some of these

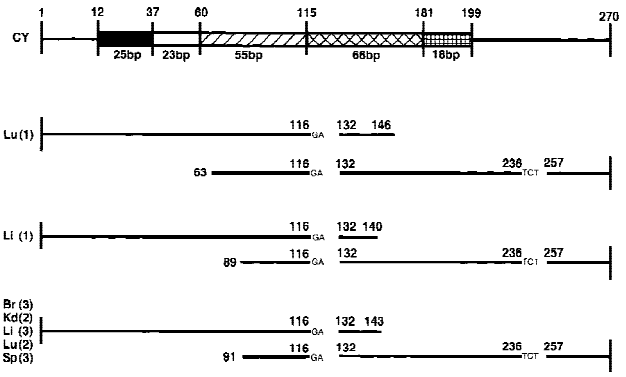

Fig. 2. Structures of the JCV(AT) TCRs derived from multiple tissues of adult PML patient AT. The diagram of the clones is interpreted as in Figure 1. Nucleotides shown at the deletion boundaries are not present in the CY TCR sequence.

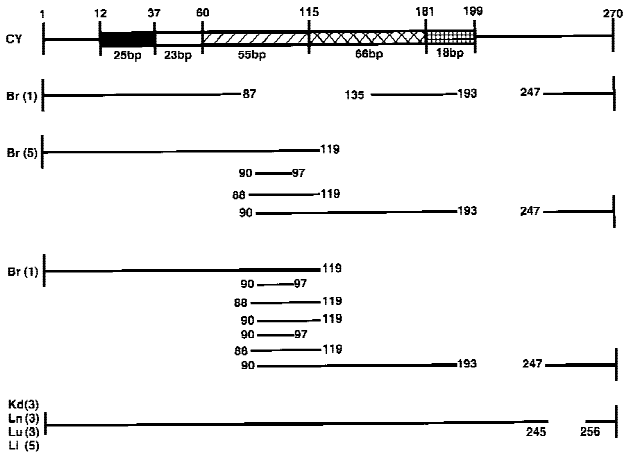

Fig. 3. Structures of the JCV(AC) TCRs derived from multiple tissues of adult PML patient AC. The diagram of the clones is interpreted as in Figure 1. Spleen (Sp) tissue was not available from this patient.

sequences are duplicated. Archetype JCV DNA was not recovered from any of the tissues available from this patient.

Some additional nucleotide changes from the CY strain were noted. A G→A transition was observed at nucleotide position 108 in all the clones sequenced from this patient. This change occurred in both copies of the promoter/enhancer, and it is also present in the Mad11Br genome [Martin et al., 1985]. In addition, Mad11Br and the new clones isolated from patient AT all had a GA addition after position 116 and a TCT addition after position 236 [Martin et al., 1985] (Fig. 2).

Identification of Archetype-Like and Rearranged Forms of JCV From Multiple Tissues of Adult PML Patient AC

Three different but related rearranged TCR clones were isolated from the brain tissue of adult PML patient AC (Fig. 3). Each clone is missing sequences between position 193 and 247. One clone had a second deletion between nucleotides 87 and 135, while the other two brain clones had multiple copies of part of this region. An archetype-like clone, having a deletion between nucleotides 245 and 256, was isolated from all other tissues available from this patient. The position of this deletion, relative to the deletions found in the three rearranged TCR clones from the brain, suggests that the latter variant did not arise from this archetype-like TCR by any obvious deletion and duplication events.

In addition to the nucleotide changes mentioned above, a small number of other nucleotide alterations in individual clones was observed relative to the CY reference strain [Yogo et al., 1990]. These base changes were at positions not recognized as sites of nucleotide polymorphism [Yogo et al., 1990], and they were considered to represent errors introduced by the Pwo/Taq DNA polymerases during PCR amplification.

Identification of JCV Genotypes by VP1 Sequence Analysis

Stoner et al. [Agostini et al., 1997c, 1998] have used nucleotide polymorphisms identified within the JCV VP1 coding region to classify the virus into genotypes 1A, 1B, 1C, 2A, 2B, 2C, and 3. To identify the JCV genotypes present in the PML patients examined in the present study, viral VP1 sequences were amplified from various tissues using the primer pair JLP15/JLP16 (Table II) [Agostini et al., 1997c]. The sequence of each clone was compared to that of the known genotypes [Agostini et al., 1997c, 1998]; alignment with the type 1A and type 2B consensus sequences is shown in Figure 4. Differences between the two consensus sequences are underlined. A type 2B genotype was identified in all the tissues from pediatric patient AH (Fig. 4A), and a type 1A genotype was recovered from all tissues from adult patient AT (Fig. 4B). Two different genotypes were isolated from the second adult patient AC. Type 2B sequences were detected in brain, and type 1A sequences were amplified from all the other tissues (Fig. 4C), suggesting that this patient had experienced a dual JCV infection. This genotype combination and tissue distribution within a single patient has recently been reported by Agostini et al. [1996a].

DISCUSSION

Our recent characterization of variant JCV genomes in a pediatric PML patient [Newman and Frisque, 1997] has altered our thinking about the trafficking of this virus within its host and about the rearrangement process that generates viruses potentially more virulent than the archetype form from which they are derived. The data suggest that rearranged forms of JCV, arising during multiplication of archetype JCV at the primary site of infection, can be transported along with archetype to multiple secondary sites by the lymphocytes. In immunocompetent hosts, viral replication may be brought under control, resulting in a persistent or latent infection of affected organs with a limited number of JCV variants. However, in immunocompromised individuals, unabated viral replication might follow the primary infection, leading to the generation of

A. JCV(AH)

Type/Clone	1753	1771	1786	1084	1818	1837	1843	1850	1869	1870
Type 1A	A	C	G	T	G	T	G	A	G	G
Type 2B	A	A	T	T	C	C	T	G	G	A
BrVP (2)	A	A	T	T	C	C	T	G	G	A
KdVP (1)	A	A	T	T	C	C	T	G	G	A
LiVP (4)	A	A	T	T	C	C	T	G	G	A
LuVP (2)	A	A	T	T	C	C	T	G	G	A
LnVP (2)	A	A	T	T	C	C	T	G	G	A
SpVP (2)	A	A	T	T	C	C	T	G	G	A

B. JCV(AT)

Type/Clone	1753	1771	1786	1084	1818	1837	1843	1850	1869	1870
Type 1A	A	C	G	T	G	T	G	A	G	G
Type 2B	A	A	T	T	C	C	T	G	G	A
BrVP(4)	A	C	G	T	G	T	G	A	G	G
KdVP (4)	A	C	G	T	G	T	G	A	G	G
LiVP (2)	A	C	G	T	G	T	G	A	G	G
LuVP (4)	A	C	G	T	G	T	G	A	G	G
SpVP (4)	A	C	G	T	G	T	G	A	G	G

C. JCV(AC)

Type/Clone	1753	1771	1786	1084	1818	1837	1843	1850	1869	1870
Type 1A	A	C	G	T	G	T	G	A	G	G
Type 2B	A	A	T	T	C	C	T	G	G	A
BrVP (5)	A	A	T	T	C	C	T	G	G	A
KdVP (3)	A	C	G	T	G	T	G	A	G	G
LiVP (4)	A	C	G	T	G	T	G	A	G	G
LuVP (3)	A	C	G	T	G	T	G	A	G	G
LnVP (3)	A	C	G	T	G	T	G	A	G	G

Fig. 4. Comparison of type 1A and type 2B consensus sequences with the VP1 sequences of clones isolated from multiple tissues. The numbers at the top of each panel indicate the nucleotide positions at which polymorphisms are known to occur in different JCV genomes. The letters *VP* following the tissue designation are an abbreviation for the VP1 late coding region, and the number in parentheses indicates the number of individual clones isolated from that tissue. Changes relative to the type 1A consensus sequence are double-underlined. Panels **A, B,** and **C** include the clones isolated from patients AH, AT, and AC, respectively.

multiple rearranged variants. In such cases, infection of the brain with some of these variants could lead to immediate pathogenic consequences.

Some aspects of this model, such as the generation of multiple rearranged variants and the early onset of PML, may only be relevant when a primary JCV infection occurs in a severely immunocompromised individual. However, other predictions of the model, including the expectation that archetype and a limited number of rearranged types of JCV would have a widespread distribution in the body, are relevant to the more common occurrence of a primary infection of an immunocompetent host.

In the current report, we have conducted a detailed analysis of the identity and distribution of JCV variants within a second pediatric PML patient and two adult PML cases to extend the initial observations made with a 5-year-old PML patient [Newman and Frisque, 1997]. At the start of this study, we had predicted that archetype and multiple rearranged JCV genomes would be detected in pediatric PML patient AH. Multiple tissues from this patient did contain JCV DNA sequences, and archetype was present in the kidney. However, multiple rearranged forms were not detected with our assay; just one rearranged TCR was recovered. It is possible that the absence of extensive rearrangement of the JCV genome in this 11-year-old patient might be the result of a functioning immune system at the time the virus was first encountered. Although the 5- and 11-year-old PML patients were both diagnosed as having SCID, the older child did receive two thymic transplants approximately 3 years prior to the onset of PML-related symptoms. Moreover, the secondary complications to his underlying immune deficiency were less severe than those observed for the younger patient (Dr. Duard Walker, personal communication). The initial exposure to the virus might have occurred earlier in the life of this 11-year-old child; in one study, 65% of children between the ages of 6 and 10 were already seropositive for JCV [Taguchi et al., 1982]. Thus, if he was able to mount a more effective response than the 5-year-old child to the primary infection, the onset of his PML at age 11 may have been the result of the reactivation of a persistent infection.

At the start of these experiments, we had anticipated that PCR analysis of adult reactivation cases of PML might yield results distinct from those obtained with pediatric cases of the disease. Previous PCR analyses of PML tissues were conducted primarily on specimens obtained from adults [White et al., 1992; Ault and Stoner, 1993; Kato et al., 1994; Agostini et al., 1997b; Elsner and Dörries, 1998], and many of our findings with adult patients AT and AC support the conclusions reached in this earlier work. For example, one predominant rearranged TCR and two minor related forms were isolated from adult patient AT, in agreement with other studies detecting one major and occasional minor variants of JCV in brain tissue [White et al., 1992; Ault and Stoner, 1993; Kato et al., 1994; Agostini et al., 1997b; Elsner and Dörries, 1998]. In our screening of this patient's tissues, we did not recover archetype JCV from the kidney. Although archetype is frequently detected in kidney tissue, there have been some reports of finding rearranged forms of JCV in the kidney of PML patients in the absence of archetype [Myers et al., 1989; White et al., 1992; Elsner and Dörries, 1998]. In the second adult PML patient, AC, sequence analysis of the VP1 region from amplified viral DNAs indicated that

this patient had suffered a dual JCV infection. Again, although this has not been a common finding, there have been other reports identifying two genotypes in a single individual [Agostini et al., 1996a, 1996b]. Furthermore, the tissue distribution of these genotypes in patient AC, i.e., type 2B in brain and type 1A in extraneural tissue, has been observed previously [Agostini et al., 1996a]. Finally, the detection of a small deletion involving nucleotides 246–255 in the archetype-like TCR amplified from patient AC (Fig. 3) has been reported for other isolates [Agostini et al., 1997b; Sugimoto et al., 1998], leading to speculation that this TCR alteration confers a replicative advantage on the viral genome.

Our analysis of the two adult patients also yielded data not reported previously in cases of reactivation PML. First, a single archetype-like JCV TCR was detected in the kidney, liver, lung, and lymph node of patient AC, indicating that this form of JCV is not confined to the kidney and urine as often reported [Yogo et al., 1990; Flægstad et al., 1991; Markowitz et al., 1991; Tominaga et al., 1992; White et al., 1992; Ault and Stoner, 1994]. It is unclear at this time whether the small deletion in this archetype TCR (nucleotides 246–255) contributes to the broad tissue distribution of this genome. Second, rearranged JCV DNAs were found to be distributed in liver, lymph node, lung, and spleen, in addition to the sites (brain, kidney, and lymphocytes) previously reported [Martin et al., 1985; Myers et al., 1989; Yogo et al., 1991b, 1994; White et al., 1992; Ault and Stoner, 1993, 1994; Dörries et al., 1994; Kato et al., 1994; Kitamura et al., 1994b; Agostini et al., 1997b; Elsner and Dörries, 1998]. Finding a positive PCR signal in all tissues examined supports the idea that the virus is transported by lymphocytes to multiple organs where it establishes a persistent or latent infection. Grinnell et al. [1983b] used Southern analysis to detect JCV DNA in the same four patients discussed here [Newman and Frisque, 1997] (Table I). Although they found the viral genome in multiple tissues of the two pediatric PML patients, detection in the two adult patients was limited to the brain (patient AT) and kidney (patient AC). Apparently, the Southern approach was too insensitive to detect the low levels of virus that had become established at other secondary sites in these two individuals.

Most of our data and those of others suggest humans are infected by a circulating archetype JCV that undergoes rearrangement of its promoter/enhancer region during replication in the host [Yogo et al., 1990; Markowitz et al., 1991; Kitamura et al., 1994a; Kunitake et al., 1995; Agostini et al., 1996b; Guo et al., 1996; Newman and Frisque, 1997; Sugimoto et al., 1997]. The simple explanation for the finding of a type 2B rearranged JCV genome in the brain of patient AC, without the corresponding detection of a type 2B archetype JCV in this individual, is that the archetype DNA was present at levels too low to be detected by our PCR cloning approach. Nevertheless, one cannot rule out the possibility that patient AC was directly infected by rearranged, rather than archetype, JCV. Recently, Elsner and Dörries [1998] suggested that rearranged forms of JCV do circulate in the population, citing as evidence the isolation of some variants, such as JCV (Mad1), from more than one individual. However, alternate explanations could be offered for this latter observation. For example, rearrangement might occur by a random mechanism, and certain rearranged variants that arise (e.g., Mad1) might have a replicative advantage; these could become the predominant form. On the other hand, the rearrangement process might exhibit a certain degree of sequence specificity, thereby yielding some rearranged TCRs more often than others. The recent detection of a type 2 JCV genome with a Mad1 TCR [Elsner and Dörries, 1998] would support either of the latter two possibilities. Because the original Mad1 variant is a type 1A genotype, the same deletion and duplication events must have occurred independently within the TCRs of two archetypes representing different genotypes.

In summary, our previous study outlined a possible series of events leading to PML in a severely immunocompromised child [Newman and Frisque, 1997]. Our present analysis of one pediatric and two adult PML patients further supports the suggestion that JCV reaches multiple tissues following a primary infection. In addition, one interpretation of this data is that only a limited number of rearranged variants become established following exposure of an immunocompetent host to archetype virus. Under these conditions, the virus may remain latent in the brain permanently, unless infection is reactivated during a subsequent impairment of the host's immune system. Finally, our data [Newman and Frisque, 1997; present study] and the data from other laboratories [Yogo et al., 1990; Markowitz et al., 1991; Kitamura et al., 1994a; Kunitake et al., 1995; Agostini et al., 1996b; Guo et al., 1996; Sugimoto et al., 1997] are most compatible with the hypothesis that humans are directly infected by archetype JCV rather than by rearranged variants.

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